

ATT 34 AMEND

13 / 0

**Claims**

1. A method for detecting binding events between specific binding pairs in which one of the pair is labelled with a nuclease enzyme, wherein the nuclease enzyme is selected from the group of nuclease enzymes that cleave a compound of formula  $RpX$  to yield  $R$  and  $pX$  and whereby the nuclease enzyme label is detected by the steps of:
- 5
- a) contacting the nuclease enzyme with a compound of formula  $RpX$ , wherein  $R$  is a 3' nucleosidyl derivative,  $p$  is a phospho radical, and  $X$  is an esterifiable moiety or, only if  $R$  is a 3' nicotinamide derivative,  $X$  is an esterifiable moiety or  $H$ , whereby  $ROH$  and  $pX$  are produced, and
- 10
- b) detecting said  $pX$  moiety or, only if  $R$  is a 3' nicotinamide derivative, detecting the  $pX$  moiety or the  $ROH$  moiety.
2. The method of claim 1 wherein  $pX$  is a prosthetic group.
- 15
3. The method of claim 2 wherein said prosthetic group is selected from the group consisting of: riboflavin 5'phosphate, pyridoxal phosphate, pyridoxamine phosphate and thiamine pyrophosphate or a derivative of any of them.
4. The method of claim 1, 2 or 3 wherein said 3'nucleoside is selected from the group consisting of adenosine, cytosine, guanine, thymidine and uridine or a derivative of any of them.
- 20
5. The method of claim 2 wherein said detecting step comprises contacting said prosthetic group with an apoenzyme.
6. The method of claim 5 wherein said apoenzyme is apoglycolate oxidase or a transaminase.
- 25
7. The method of claim 1 wherein  $X$  is a 1,2-dioxetane compound.
8. The method of claim 7 wherein said detecting step comprises contacting said 1,2-dioxetane phosphate with a phosphatase enzyme, whereby light is produced, and detecting the light produced.

ART 34 AMDT

9. The method of any preceding <sup>14</sup> / 1 claim, wherein the nuclease enzyme is selected from enzymes of the class EC.3.1.30.1.
10. The method of claim 9 wherein the nuclease enzyme is selected from the group consisting of nuclease P1, nuclease S1 and mung bean nuclease.
- 5 11. The method of any preceding claim, wherein the method is applied to detecting binding events between specific binding pairs selected from the group consisting of: An antibody and a hapten or antigen; a nucleic acid probe and its corresponding target sequence; a biotin derivative and avidin, streptavidin or neutravidin; and a ligand and a receptor.
- 10 12. A method for detecting binding events between specific binding pairs in which one of the pair is labelled with a nuclease enzyme, wherein the nuclease enzyme is selected from the group of nuclease enzymes that cleave a compound of formula  $RpX$  to yield  $R$  and  $pX$  and whereby the nuclease enzyme label is detected by the steps of:
- 15 a) contacting the nuclease enzyme with a phosphodiester comprising a prosthetic group and a 3'nucleoside, whereby said prosthetic group is produced, and
- b) detecting said prosthetic group.
- 20 13. A method for detecting binding events between specific binding pairs in which one of the pair is labelled with a nuclease enzyme, wherein the nuclease enzyme is selected from the group of nuclease enzymes that cleave a compound of formula  $RpX$  to yield  $R$  and  $pX$  and whereby the nuclease enzyme label is detected by the steps:
- 25 a) contacting the nuclease enzyme with a compound of formula  $RpX$ , wherein  $R$  is a 3'nicotinamide derivative,  $p$  is a phospho radical, and  $X$  is an esterifiable moiety, whereby  $ROH$  and  $pX$  are produced, and
- b) detecting said  $ROH$  moiety.
14. The method of claim 13 wherein said nicotinamide derivative is  $NAD$  or  $NADH$ .
- 30 15. The method of claim 13 or 14 wherein said detecting step comprises conducting enzymatic cycling of  $NAD$ - $NADH$  interconversions in the presence of a dehydrogenase, a substrate for said dehydrogenase, a tetrazolium dye and a

Art 34 Amdt

15 18  
diaphorase, and detecting the amount of the NAD or NADH with a colour-development signal of formazan which is produced by the action of diaphorase and NADH-NAD conversions.

16. A kit for detecting binding events between specific binding pairs in which one  
5 of the pair is labelled with a nuclease enzyme, wherein the nuclease enzyme is selected from the group of nuclease enzymes that cleave a compound of formula RpX to yield R and pX and whereby the nuclease enzyme label is detected, the kit comprising:

- 10 (a) a compound of formula RpX, wherein R is a 3'nucleosidyl derivative, p is a phospho radical, and X is an esterifiable moiety or, only if R is a 3' nicotinamide derivative, X is an esterifiable moiety or H, whereby ROH and pX are produced and
- 15 (b) a detection system for detecting pX or, only if R is a 3' nicotinamide derivative, a detection system for detecting the pX moiety or for detecting the ROH moiety.

17. The kit of claim 16 wherein RpX is NAD3P or NAD3PH.

18. The kit of claim 16 wherein RpX is a nucleoside-3'phosphoriboflavin derivative.

20 19. The kit of claim 16 wherein RpX is a nucleoside-3'-phospho-pyridoxal derivative.

20. The kit of claim 16 wherein RpX is a nucleoside-3'-phospho-pyridoxamine derivative.

25 21. The kit of claim 16 wherein RpX is a nucleoside-3'-phospho-thiamine derivative.

22. The kit of claim 16 wherein RpX is a nucleoside-3'-phospho-1,2-dioxetane derivative.

**ART 34 AMDT**

23. The kit of claim 16 wherein said <sup>13</sup> detection system comprises a dehydrogenase, a diaphorase, and a tetrazolium compound.
24. The kit of claim 16 wherein said detection system comprises an apoenzyme.
25. The kit of claim 16 wherein said detection system comprises a phosphatase.